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## Guidelines for Research Involving Viral Vectors: Alphaviruses and Alphavirus Vectors

Alphaviruses (family *Togaviridae*) are enveloped, positive-sense, single-stranded RNA viruses that are transmitted by mosquitoes. They are members of the *Togaviridae* family, which also includes the *Reoviridae* and *Rotaviridae* families. Alphaviruses are characterized by their icosahedral capsids and lipid bilayer envelopes. The genome consists of a single strand of RNA with a 5' cap and a 3' poly(A) tail. The genome encodes for three structural proteins (C, E1, and E2) and two non-structural proteins (NS1 and NS2). The C protein is the capsid protein, E1 is the glycoprotein that forms the envelope, and E2 is another glycoprotein. NS1 and NS2 are non-structural proteins that play roles in viral replication and evasion of the host immune response. Alphaviruses are known to cause diseases such as Chikungunya fever, Eastern equine encephalomyelitis (EEE), and Sindbis fever. Research involving viral vectors, particularly alphavirus vectors, is used for gene delivery and vaccine development. This document provides guidelines for the safe and effective use of these vectors in research settings.

an amplifying host for some alphaviruses, such as certain strains of VEEV, and can infect mosquitoes when fed upon. VEEV, EEEV and WEEV have been demonstrated to be infectious via the aerosol route when delivered in high concentrations in the laboratory setting.

## Laboratory Acquired Infections

Laboratory acquired infections have been documented for some alphavirus including at least two deaths. Exposure to aerosols, contact with broken skin or contaminated animal bedding and accidental autoinoculation

- x All activities with infectious material should be conducted in a biological safety cabinet (BSC) or other appropriate primary containment device in combination with personal protective equipment.
- x Centrifugation of infected materials must be carried out in closed containers placed in sealed safety cups, or in rotors that are loaded or unloaded in a biological safety cabinet.
- x Mouth pipetting is strictly prohibited mechanical pipetting devices will be used. Pipettes should have capped/plugged tops. Pipette tips may or may not require filters depending on the biological or application. Care must be taken to not contaminate hand-held or automatic pipettors and other instruments with toxic or infectious materials.
- x Biohazard markings will be on all contaminated waste and waste disposal containers in addition to any equipment used for work or for storage of biological hazards
- x

## Personal Protective Equipment

Personal protective equipment for BSL<sub>2</sub> laboratory work includes a dedicated laboratory coat, eye protection, and disposable gloves at a minimum.

Personnel entering the BSL<sub>2</sub> laboratory should remove street clothing and jewelry; and change into dedicated laboratory clothing and shoes before donning two pairs of booties, two pairs of gloves and respiratory protection (i.e. PAPR unit). Additional protection must be worn over

## Recombinant Alphavirus Research

All protocols involving recombinant alphavirus vectors, regardless of the funding source, must comply with the NIH Guidelines for Research Involving Recombinant and Synthetic Nucleic Acid; and must be approved by the Institutional Biosafety Committee before any work is initiated.

### Employee Exposure

**Eye Exposure** remove PPE if necessary, proceed to the eyewash station in the laboratory, and rinse eyes with cold running water for 15 min.

**Skin Exposure** remove PPE if necessary. If there are no cuts or abrasions, wash the affected area with soap and water.

**Accidental Needlestick Injury** or exposure of cuts/abrasions remove PPE and wash the affected area with soap in the laboratory sink. Allow the wound to bleed if bleeding is not excessive and irrigate the wound for 15 min.

**Report Incidents and Seek Treatment** actual or suspected exposure incidents should be reported immediately to the supervisor and biological safety officer. The responsible official (RO) and/or alternate responsible official (ARO) should also be notified in the event of injuries/exposures involving **CR3** agents. The individual should go to the University Physicians Group (normal working hours) or to the emergency room at USA Medical Center. The attending physician will arrange consultation by a USA infectious disease physician. If the injury/exposure involves a select agent, the RO/ARO will immediately notify the CDC and follow up with a Form 3 within seven calendar days.

### Spills and Disposal Procedures

- x The use of additional PPE beyond what is normally worn on entry to the laboratory will be determined during the risk assessment and communicated to the response team prior to cleanup.
- x In most situations the spill can be handled as follows:
  - o Add premeasured decontaminant (e.g. Bleach, Roccal, ~~Moban~~ Plus) to the water provided in the spill kit.
  - o Gently cover the spill with paper towels or ~~RZ~~ powder.
  - o Apply the decontaminant solution starting ~~at~~ the perimeter of the spill and working towards the center.
  - o Allow 30 minutes' contact time with the decontaminant solution before cleanup, except in emergencies (i.e. injury).
  - o Remove paper towels or ~~RZ~~ to a biohazard bag along with any paper towels ~~used~~ to wipe the area dry. If the decontaminant solution was used on metal, wipe the area with 70% ethanol.
  - o Discard protective clothing into the biohazard bag and autoclave.
- x Additional procedures/decontamination (chemical decontamination of surfaces or VHP) will be determined during the risk assessment and communicated to the response team as necessary.
- x Confirm that the spill has been reported, and that the ~~clean~~ and all necessary paperwork have been completed.
- x If the spill involves ~~R3~~ select agents, ~~lab~~ personnel in the vicinity of spill at the time of occurrence will be required to give a formal report to the RO/ARO who in turn will notify the CDC.

Small spills (less than 1 ml) within a BSC can be handled by covering the spill with a paper towel soaked in disinfectant and allowing an appropriate contact time before collecting the paper towel and processing in the normal waste stream. For larger spills, the following steps must be taken.

- x Stop what you are doing and secure any remaining stocks in the cabinet. It is never appropriate to continue working in a BSC that is grossly contaminated with infectious agents.
- x If working with ~~R3~~ pathogens, remove wrap around gown into the BSC or tear off arms into the BSC. If working with ~~R2~~ pathogens decontaminate sleeves of laboratory coat by saturating with appropriate disinfectant before exiting cabinet.
- x Remove outer gloves and exit the BSC.
- x Replace outer gloves
- x Report the spill to the supervisor and the RO/ARO if ~~R3~~ viruses are involved. Allow the air to settle in the BSC (approximately 10 min.)
- x Don a new PPE and return to the BSC with additional cleaning supplies.
- x Cover the spill with paper towels and pour decontamination solution over the paper towels working from the outside towards the center. Never use a spray bottle to apply decontamination solution after a spill.
- x Use a paper towel soaked in decontamination solution to wipe down any other items in the BSC and allow 30 min. contact time for the decontamination solution to work.
- x After the appropriate contact time, bag all waste and remove all items from the BSC,

what equipment was in the hood these might need to be bagged for storage until a